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FULL PAPER

Effect of chitosan addition on proximate composition, antioxidant activity, and sensory acceptance of biscuits containing red and purple roselle extract

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Abstract

The purpose of this research was to produce biscuits containing red and purple roselle extract with the addition of chitosan to improve the biscuits' proximate composition, total phenolic, antioxidant activity, and sensory acceptance. The research used a completely randomized design with seven factors containing red and purple roselle extract, and chitosan, respectively; C1 (0:0:1)%; C2 (10:0:0)%; C3(0:10:0) %; F1(4:6:1)%; F2 (6:4:1)%; F3(4:6:2)%; and F4(6:4:2)%. Proximate composition (moisture, protein, fat, carbohydrate, ash and fibre) was determined using standard methods. The total phenolic content of roselle extract biscuits was measured using the Folin-Ciocalteau method, whereas the determination of antioxidant activity was carried out by applying the 2,2-Diphenylpicrylhydrazyl (DPPH) method. The proximate analysis of the biscuits produced high moisture content ranging from 3.25 to 3.94% W/W, protein content from 6.68 to 7.43% W/W, and fat content (19.13-19.74% W/W). The ash content varied from 1.62 to 1.72% W/W, fibre content ranged from 3.32 to 5.06% W/W, and carbohydrate contents were 62.50-65.40% W/W. The concentration of 1% chitosan in the biscuit formulation was also more effective in increasing the phenolic content and antioxidant activity (IC_{50}), respectively 3.36 mg/100 g and 71.81 ppm in the F1 biscuits samples (red roselle 4% and purple 6%). Similarly, in F1 with the addition of 1% chitosan to sample biscuits containing red (4%) and purple (6%) roselle extract, the panellists received good acceptance of the aroma, colour, texture, taste, and overall biscuits made. Biscuits containing 6% purple roselle and 1% chitosan in the formulation could be provided with a significant contribution to increasing total phenol, antioxidant activity, and sensory acceptance compared to the others. Therefore, roselle extract and chitosan can be used as additional biscuit supplements that can increase nutritional content and antioxidant activity.

1. Introduction

Biscuits are sweet and small snacks that are inserted into pastries with low water content. This snack is a type of bakery food that is suitable for people of all ages (Adedara and Taylor, 2021). Its physical composition is also ready to eat, have a longer shelf life, has good proximate composition and have a variety of taste (Fathonah *et al*., 2020). Biscuit-making ingredients in general are still dominated by the use of serialia from grains as the main component. However, only a small amount of non-serialia material is used, therefore biscuits only contain a small amount of dietary fibre. Efforts to diversify biscuit products need to be considered to increase the value of dietary fibre and taste variations. On the other hand, the biscuit formula is also easy to modify during the manufacturing process, so it is possible to make the formulation with the addition of ingredients other than serialia. One of the ingredients for making biscuits from other than serialia which has a high food content is roselle (*Hibiscus sabdariffa*) (Zannou *et al*., 2020).

Roselle (*Hibiscus sabdariffa*) is a plant in the *Malvacea* family that grows wild in tropical climates. There are two types of roselle varieties in Indonesia, namely red and purple. The bioactive content in roselle

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can be efficacious as traditional medicine, food colouring, antioxidants and preservatives (Andzi Barhé and Feuya Tchouya, 2016; Juhari *et al.*, 2018). Roselle calyces are very beneficial for health because they contain phenolic compounds that functioned as antioxidants that can cure degenerative diseases including lowering blood cholesterol activity, anticancer, reducing blood pressure, and antidiabetic (Cittan *et al*., 2018; Wu *et al*., 2018). The content of roselle bioactive compounds as antioxidants is often used in the manufacture of mixed and herbal drinks (Kamdaeng and Singkaew, 2021). However, the use of roselle petals has not been applied in the manufacture of small snacks such as biscuits. Roselle calyces can be used as the basis of functional biscuit innovations because they can be processed and diversified into extracts. The provision of roselle extract in biscuits can reduce micronutrient deficiencies in foodstuffs, and increase both the variety of taste and the value of biscuits. The addition of roselle in biscuit products could diversify food sources rich in antioxidants (Jabeur *et al*., 2017).

Food ingredients other than roselle have high antioxidant activity, namely chitosan. Chitosan has a high economic value and its processed products can be used for various purposes. Not only chitosan can be used in food processing, medicine and biotechnology, but it is also an attractive material in pharmaceutical biomedical applications, environmental wastewater treatment, and agriculture (Hossain and Iqbal, 2014; Afonso *et al*., 2019). The nature of chitosan is not toxic, has biological activity, biocompatibility, biodegradability, and can be modified chemically and physically (Abd El-Hack *et al*., 2020; Rasul *et al*., 2020). There have been many studies on chitosan as a preservative for food products such as processed fish, fruits, juice purifiers, and edible forming because of its antioxidant and antimicrobial activity (Wei *et al*., 2019). However, the use of chitosan in additional supplements in functional biscuits containing red and purple roselle has not been studied previously.

Chitosan is a positively charged polysaccharide compound that is very effective in absorbing anions from organic substances such as fats and proteins due to the presence of the polymer chain, amino acid and hydroxyl groups (Tokatlı and Demirdöven, 2018). Chitosan in addition to being developed in various fields is also used as an elicitor. Chitosan in its work can induce and increase the content of secondary metabolites such as saponins and polyphenols, therefore it can activate and improve the antioxidant system and eliminate oxidative damage (Qiu *et al*., 2021). Chitosan can improve the appearance and texture of a product as well because it can bind the tannin content and is heat resistant (Agnolucci *et al*., 2020).

Thereby, this study was intended to evaluate the proximate composition, total phenolic and antioxidant activity, as well as to assess the sensory acceptance of biscuits containing red and purple roselle extract with the addition of chitosan. The provision of chitosan in biscuit preparations was also expected to increase the proximate value, total phenolic, and antioxidant activity as well as the value of sensory acceptance.

2. Materials and methods

Red and purple roselle calyces were obtained from roselle farmers in Malang Regency, East Java. The chitosan used in this study was commercial chitosan which was purchased at the CHIM-Pharmacy Multipurpose Store Bandung-Indonesia with a deacetylation degree of 80.5% and a pH of 6.7. All other additives such as wheat flour, skim milk, egg yolk, margarine, cornstarch, and baking soda were purchased at the local supermarket Carrefour Tegal City. All analytical materials were sodium hydroxide, potassium sulfate, hydrochloric acid, hexane, methyl red-methylene blue indicator, distilled water, mercury (II) oxide, boric acid, sulfuric acid, 95% ethanol, iodine solution, phosphate buffer pH 6 and pH 7, and acetate solution. In addition, the study was equipped with a spectrophotometer, a magnetic stirrer, a 50 mesh sieve, oven, desiccator, and analytic Ohaus (NV2201).

2.1 Red and purple roselle extract preparation

The stages of making roselle flower extract begin with the process of washing the roselle flower petals with distilled water and then drying them under sunlight. The dried roselle petals were placed into the maceration container and then soaked in distilled water until all parts of the simplicia were moistened. The position maceration container was covered with aluminium foil, then stored for 24 hrs in a place away from sunlight and occasionally stirred to speed up the extraction process. Next, the filtrates were filtered and separated from the dregs using gauze (Safdar *et al*., 2017). The filtrates were put in a petri dish and then placed in the refrigerator for the freezing process. After freezing, the sample was put into a freeze-drying device at a temperature of -30°C for 24 hrs to obtain roselle flower extract.

2.2 Biscuit preparation

The formulation of biscuits containing roselle extract with the addition of chitosan was presented in Table 1. The procedure for making biscuits began by mixing margarine, sugar, and developer ingredients to form a homogeneous cream using a mixer. Next, the egg yolks were added and beaten at low speed, and skim milk was added during the cream production process. The last

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Table 1. The formulation of biscuits containing roselle extract and chitosan

Ingredients	C1/control	C2/control	C3/control	F1	F2	F3	F4
Wheat flour (g)	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Red roselle(g)	0.0	10.0	0.0	4.0	6.0	4.0	6.0
Purple roselle (g)	0.0	0.0	10.0	6.0	4.0	6.0	4.0
Chitosan (g)	1.0	0.0	0.0	1.0	1.0	2.0	2.0
Skim milk (g)	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Sugar (g)	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Margarine (g)	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Cornstarch (g)	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Egg yolk (g)	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Baking powder (g)	2.0	2.0	2.0	2.0	2.0	2.0	2.0

C1: red/purple roselle/chitosan (0:0:1)%, C2: red/purple roselle/chitosan (10:0:0)%, C3: red/purple roselle/chitosan (0:10:0)%, F1: red/purple roselle/chitosan (4:6:1)%, F2: red/purple roselle /chitosan (6:4:1)%, F3: red/purple roselle//chitosan (4:6:2)%, F4: red/purple roselle//chitosan (6:4:2)%.

stage was adding flour and stirring the mixture by hand, followed by the addition of red and purple roselle extract and chitosan powder. The stirring process was carried out until the dough expanded and became smooth. The biscuit baking process was carried out at 150°C for 25 mins. This method was a modification of making biscuits by Choudhury *et al*. (2015).

The addition of chitosan in the biscuit formulation was 1% and 2% of the amount of wheat flour (100 g) used in the dough, *i.e*. 1 g and 2 g, respectively. The use of 1 g and 2 g chitosan refers to the study conducted by Zhang *et al*. (2018) in the manufacture of edible coatings based on chitosan. The roselle extract used was 4%, 6%, and 10% of the amount of wheat flour in the dough, i.e. 4 g, 6 g, and 10 g, respectively. The provision of roselle extract as much as 4 to 10 g in the biscuit formulation also refers to the study by Zannou *et al.* (2020) on the manufacture of hot and cold drinks. A completely randomized design was used in the study, with seven factors containing red, purple roselle extract, and chitosan, respectively; C1 (0:0:1)%; C2 (10:0:0)%; C3 (0:10:0) %; F1 (4:6:1)%; F2 (6:4:1)%; F3 (4:6:2)%; and F4 (6:4:2)%.

2.3 Proximate composition

Determination of the proximate composition was carried out using the official standard method from the Association of Official Analytical Chemists (AOAC) (Latimer Jr., 2016). The proximate parameters analysed were moisture, protein, fat, carbohydrates, ash, and fibre (Kumoro *et al*., 2020).

2.3.1 Moisture content

A total of 3 g of sample (W1) was placed on a cup and dried in an oven for 5 hrs at a temperature of 100–110°C until dry. Then the cup was moved into a desiccator until it cooled down, and then the sample was weighed again (W2)(Kamdaeng and Singkaew, 2021). The water content was calculated as follows.

$$\text{Moisture}(\%) = \frac{\text{W1-W2}}{\text{W1}} \times 100\%$$

Where, W1 = sample weight before drying and W2 = sample weight after drying.

2.3.2 Ash content

The determination of ash content was conducted by placing 1 g of sample into a silica container. The sample was spread evenly in layers and put into a muffle furnace. The combustion temperature in the furnace reached 550°C for 2 hrs until the sample became slightly grey or white (Liu *et al*., 2015). The ash content can be calculated by the formula:

$$\text{Ash}(\%) = \frac{\text{W2-W1}}{\text{Weight of the sample}} \times 100\%$$

Where, W1 = container weight plus residue and W2 = empty container weight.

2.3.3 Fat content

The determination of fat content was done using the Soxhlet extraction method. The pumpkin was first weighed as its initial weight. A total of 2 mL of the sample was placed in 200 mL of chloroform into the Soxhlet apparatus. The flask was then baked at 110°C for 2 hrs before being cooled in a desiccator (Koczoń *et al*., 2016). The formula for calculating crude fat content can be seen as follows.

$$\text{Fat}(\%) = \frac{\text{W2-W1}}{\text{Weight of the sample}} \times 100\%$$

Where, W1 = the weight of the extraction flask and W2 = the weight of the extraction flask plus the weight of the dried crude fat.

2.3.4 Protein content

The determination of protein content in samples was done by the Kjeldahl method according to Dusuki *et al*. (2020) with slight modifications. Samples of 2 g were

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placed in a Kjeldhal flask and two catalyst tablets and 20 mL of concentrated sulfuric acid were added. The sample solution was put in a steam distillation device at 400°C for 80 mins and 50 mL of distilled water was added for dilution. Then 10 N concentrated sodium hydroxide solution was poured in gradually until the sample solution was alkaline. The container solution was mixed with 50 mL of a 2% boric acid solution and 5 drops of methyl red indicator in a beaker. A standard 0.1 N hydrochloric acid solution was used to titrate the reservoir solution until the colour returned to pink (Wang *et al*., 2016).

2.3.5 Dietary fibre content

A total of 2 g of the material was placed in a flask and stirred for 30 mins with 50 mL of 1% sulfuric acid. The mixture was then added with 50 mL of 3% sodium hydroxide and then brought to a boil. Next, it was filtered with Whatman paper on a Buchner funnel and washed with 1% sulfuric acid, 96% ethanol, and hot water (Danhassan *et al*., 2018). The amount of residue on Whatman paper was then weighed.

$$\text{Fibre (\%)} = \frac{W1\text{-}W2}{W1} \times 100\%$$

Where, W1 = initial weight of sample and W2 = sample final weight

2.3.6 Carbohydrate content

Measurement of total carbohydrate levels used the carbohydrate by difference of the AOAC method (Latimer Jr, 2016). Total carbohydrate content = 100%−[Moisture(%) + protein(%) + fat(%) + ash(%) + fiber (%)](Kumoro *et al*., 2020).

2.4 Total phenolic content

The Folin-Ciocalteau method was used to determine the total phenolic content of biscuits (Mahloko *et al*., 2019) with a few modifications. A total of 10 mg of sample was added to 10 mL of methanol to the 10 mL mark in the volumetric flask, and then it was centrifuged. An amount of 0.5 mL of 50 ppm gallic acid solution was pipetted to 5 mL of Folin-Ciocalteau reagent solution (10% v/v) and 2 mL of 10% (w/v) sodium carbonate solution. The sample was incubated for 30 mins at room temperature (25°C) in the dark to observe its development, which revealed a blue colour shift. The absorbance was measured using a UV-Vis Spectrophotometer at 650 nm. Gallic acid standards and calibration curves were also prepared according to the procedure from various concentrations of gallic acid (20, 40, 60, 80, and 100 ppm). The total phenol content was expressed as mg gallic acid equivalent (GAE)/100 g extract.

2.5 Antioxidant activity

The antioxidant activity of biscuit samples was determined by spectrophotometric reduction of the 2,2-Diphenylpicrylhydrazyl (DPPH) radical (Patrignani *et al*., 2016). A sample of 2 g was diluted in a methanol solution and stirred. The sample concentrations were prepared (20, 40. 60, 80, and 100 ppm) and diluted with 5 mL of methanol solution, and then 1 mL of 1 mM DPPH solution was added. The mixture was mixed before being incubated at room temperature for 30 mins in the dark. Readings were taken at 517 nm. The percentage of free DPPH radical scavenging activity that was suppressed was calculated.

$$\text{Inhibition (\%)} = \frac{\text{Acontrol-Asample}}{\text{Acontrol}} \times 100\%$$

The concentration of the sample can provide inhibition of 50% (IC_{50}) and the value of IC_{50} was calculated by estimating using the fitted line (Laela *et al*., 2021). The IC_{50} value was calculated graphically by linear regression using a line plotted on a curve. The following formula was used to compute the IC_{50} of the sample.

2.6 Sensory evaluation

The sensory acceptance test was carried out at the Department of Pharmacy, Polytechnic Harapan Bersama, Tegal, Indonesia by twenty panellists. The testing process was conducted by presenting biscuits without chitosan and roselle extract as well as biscuits with the addition of chitosan and roselle extract at various proportions randomly and using a certain code. The code chosen did not provide any clues about the test to the panellists. After sample testing, panellists were then asked to drink water that had been provided by the researcher before trying the next sample. Some of the acceptance of sensory that were evaluated on the rating of the degree of preference included aspects of aroma, colour, texture, taste, and overall acceptance. The sensory acceptance of the sample was evaluated on a hedonic scale with a value category of 7 points (1 = strongly dislike; 4 = dislike or neither like; 7 = strongly like) (Choudhury *et al.*, 2015).

2.7 Statistical analysis

All the results were expressed as mean±standard deviation of triplicates in three independent experiments. Proximate composition, total phenolic, and antioxidant activity data were assessed by one-way analysis of variance (ANOVA) where the mean values were considered to be significantly different when $p<0.05$ was obtained using the Statistical Package for Social Sciences (SPSS) software. Sensory reception data used the Kruskal-Wallis Non-Parametric test and showed

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significance at $p<0.05$ if the mean values were predicted to be different.

3. Results and discussion

3.1 Proximate composition

Table 2 shows the moisture content of the biscuit samples containing roselle extract with the addition of 1% and 2% chitosan at C1 and F4, i.e. 3.87±0.01% and 3.37±0.23% W/W, respectively. The F4 biscuit samples with the addition of 2% chitosan concentration showed a significant difference ($p<0.05$) between each other. This could be possible since chitosan has a hydrophobic composition, thereby reducing the amount of moisture content in biscuits. A study conducted by Yuan *et al*. (2016) explained that chitosan reduces the content and velocity of water vapour in edible films and coatings.

Table 2 shows that the protein and fat levels in the F3 biscuit samples (2% chitosan and 6% purple roselle extract) were significantly different ($p<0.05$), which were 7.47±0.15% and 19.88±0.05% W/W, respectively compared to the others. The increase in protein and fat levels was due to chitosan being a polysaccharide compound that had a positive charge and amino acid groups along the polymer chain, it absorbs the anion content of organic substances such as fats and proteins (Wang *et al*., 2016). The protein and fat content of the sample biscuits with 6% purple roselle extract had higher levels of activity compared to 6% red roselle extract in the formulation. This study was also in line with Ningrum *et al.* (2019), showing that purple roselle flowers contained 16 volatile organic acids and red roselle had 11 volatile organic acids. A related research by Aryanti *et al*. (2019) also showed that the anthocyanin content in purple roselle (75.60 mg/L) was higher than that of red roselle (63.45 mg/L).

The ash content in the biscuit samples with the addition of 1% chitosan in F1 (4% red roselle and 6% purple) showed a significant value ($p<0.05$) with the highest number recorded at 1.88±0.03% W/W. It could be assumed that chitosan as an elicitor can absorb secondary metabolites, organic acids, and minerals contained in red and purple roselle. The carbohydrate content in the biscuit samples also decreased, this was due to other changes in the composition of the biscuits. Based on the equation by difference formula, carbohydrate levels are strongly influenced by other nutrients. The higher the value of carbohydrates, the lower the proximate components (moisture, protein, fat, and ash). Dietary fibre content was determined by the amount of water content and water activity, where a larger amount of water was required gradually in the process of making biscuit dough containing large amounts of dietary fibre (Matsuo *et al.*, 2019).

3.2 Total phenolic

Table 3 indicates the total phenolic content of biscuits samples with the addition of 1% chitosan in F1 showed a significant difference ($p<0.05$) at 3.36±0.05 mg/100 g compared to other samples. Several studies have also reported that elicitation of chitosan can contribute to the increased production of various secondary metabolites such as saponins, triterpenoids, and flavonoids (Lucini *et al*., 2018; Qiu *et al.*, 2021). The total phenolic yield with the addition of 2% chitosan decreased in the sample biscuits at F3 and F4 by

Table 2. Proximate composition of biscuits

Biscuit Formulation	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)	Fiber (%)
C1/control	3.87±0.01	6.68±0.05	19.13±0.03	1.62±0.02	65.40±0.12	3.32±0.15
C2/control	3.94±0.03	7.16±0.03	18.74±0.11	1.67±0.22	63.14±0.33	5.35±1.1
C3/control	3.91±0.12	7.18±0.02	18.85±0.02	1.69±0.04	63.15±0.47	5.52±0.33
F1	3.75±0.05	7.33±0.22	19.08±0.22	1.88±0.03	61.70±0.15	6.51±0.02
F2	3.67±0.01	7.29±0.12	19.02±0.14	1.83±0.01	61.47±0.12	6.48±0.08
F3	3.25±0.02	7.47±0.15	19.88±0.05	1.76±0.05	62.74±0.14	5.04±0.05
F4	3.37±0.23	7.43±0.23	19.74±0.03	1.72±0.12	62.50±0.05	5.06±0.06

All values represent the mean±SD of three replicates. C1: red/purple roselle/chitosan (0:0:1)%, C2: red/purple roselle/chitosan (10:0:0)%, C3: red/purple roselle/chitosan (0:10:0)%, F1: red/purple roselle/chitosan (4:6:1)%, F2: red/purple roselle /chitosan (6:4:1)%, F3: red/purple roselle//chitosan (4:6:2)%, F4: red/purple roselle//chitosan (6:4:2)%.

Table 3. Total phenolic, antioxidant activity, and IC_{50} value of biscuits

	C1/control	C2/control	C3/control	F1	F2	F3	F4
Phenolic (mg/100 g)	1.87±0.03	2.14±0.02	2.45±0.14	3.36±0.05	3.08±0.13	2.42±0.04	2.38±0.10
Antioxidant activity (%)	42.12±0.02	44.14±0.01	45.76±0.13	50.52±0.02	49.38±0.21	47.42±0.08	46.28±0.17
IC_{50} value (ppm)	98.64±0.04	86.24±0.02	81.56±0.03	71.81±0.11	75.78±0.05	95.80±0.02	97.26±0.22

All values represent the mean±SD of three replicates. C1: red/purple roselle/chitosan (0:0:1)%, C2: red/purple roselle/chitosan (10:0:0)%, C3: red/purple roselle/chitosan (0:10:0)%, F1: red/purple roselle/chitosan (4:6:1)%, F2: red/purple roselle /chitosan (6:4:1)%, F3: red/purple roselle//chitosan (4:6:2)%, F4: red/purple roselle//chitosan (6:4:2)%.

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2.42±0.17 and 2.38±0.10 mg/100 g, respectively. The decrease in the amount of phenolic might be caused by the elicitor component that recognizes all receptors will experience a saturation point, the addition of the given eliminator molecules cannot increase the amount of phenolic secondary metabolites. A study conducted by Jiao *et al*. (2018) also reported that the addition of chitosan concentrations of 150 mg/L and 200 mg/L makes it more effective in producing greater flavonoid content than 400 mg/L chitosan.

3.3 Antioxidant activity

Table 3 shows the effect of giving chitosan at different concentration levels on the DPPH radical scavenging activities of the biscuit formulation. Biscuit samples in F1 (6% purple and 1% chitosan) showed significant values ($p<0.05$) with the highest IC_{50} value of 71.81±0.11 ppm, followed by F2 of 75.78±0.05 ppm, and the smallest in control C1 of 98.64±0.04 ppm. The antioxidant activity of the sample is highly dependent on the number of phenolic compounds and other phenolic compounds produced from non-enzymatic browning reactions during the process (Karseno *et al*., 2018).

Other studies also showed that chitosan was able to increase the antioxidant value and stability of electrostatic interactions compared to without chitosan in beverage preparations (Hu *et al.*, 2020). The biscuit formulation with the addition of purple roselle also showed greater antioxidant activity compared to biscuits with red roselle ingredients. This study was supported by research that showed the antioxidant activity of purple roselle was greater than that of red roselle (Kusnadi and Purgiyanti, 2021). The antioxidant activity and IC_{50} value have a negative correlation value, the lower the IC_{50} value indicates the higher the antioxidant activity of the sample (Ait Lahcen *et al.*, 2020). The increase in antioxidant activity of biscuit samples could be caused by the formation of more melanoidin compounds (Karrar *et al*., 2021).

3.4 Sensory acceptability of biscuits containing roselle extract and chitosan

Sensory acceptance of biscuits containing roselle extract with the addition of chitosan was used to determine the acceptability of various sensory compositions of biscuits such as colour, aroma, taste, texture, and overall. The purpose of the product sensory test was to provide an objective acceptance of whether a product is favourable or not (Silva *et al*., 2018). Table 4 indicated that 1% chitosan in F1 showed a significant difference ($p<0.05$) in the panelists' preference level of 5.44±0.05 compared to others. The addition of 1% chitosan, red roselle extract (4% and 6%) and purple (4% and 6%) in F1 and F2 had good acceptance by the panelists of the aroma produced by the biscuits.

In Figure 1 of the biscuit samples, the panellists gave a better acceptance of the colour, the biscuits with the addition of 1% chitosan were highly favoured by the panellists because they had a brighter colour than the others. Chitosan could bind tannin, thus the resulting biscuit colour was brighter than the sample without chitosan. The higher concentration of chitosan bound the tannins and other pigments in the biscuit dough, therefore the colour looked pale and reduced the panellist's acceptance (Lu *et al*., 2016; Agnolucci *et al*., 2020). Likewise, the acceptance of taste and texture with the addition of 1% chitosan, red roselle extract (4% and 6%), and purple (4% and 6%) in F1 and F2 had the highest values compared to the other formulas.

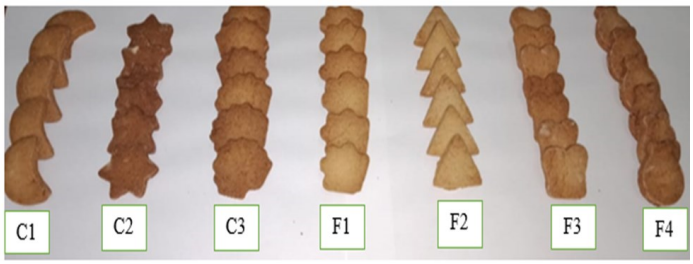

Figure 1. Biscuit products containing rosella extract and chitosan.

Table 4. Sensory evaluation of the biscuits

Biscuit formulation	Aroma	Colour	Texture	Taste	Overall
C1/control	5.05±0.01	4.91±0.05	5.10±0.03	4.52±0.01	4.73±0.01
C2/control	4.80±0.03	4.36±0.01	4.80±0.08	4.24±0.05	4.66±0.05
C3/control	4.98±0.02	4.68±0.02	4.98±0.12	4.63±0.15	4.64±0.03
F1	5.44±0.05	5.53±0.03	5.50±0.01	4.83±0.11	5.48±0.04
F2	5.33±0.01	5.20±0.11	5.43±0.04	4.84±0.03	5.36±0.02
F3	4.88±0.02	4.78±0.04	5.02±0.03	4.62±0.01	4.96±0.21
F4	4.75±0.23	4.86±0.03	5.07±0.02	4.75±0.04	4.92±0.05

All values represent the mean±SD of three replicates. C1: red/purple roselle/chitosan (0:0:1)%, C2: red/purple roselle/chitosan (10:0:0)%, C3: red/purple roselle/chitosan (0:10:0)%, F1: red/purple roselle/chitosan (4:6:1)%, F2: red/purple roselle /chitosan (6:4:1)%, F3: red/purple roselle//chitosan (4:6:2)%, F4: red/purple roselle//chitosan (6:4:2)%.

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4. Conclusion

The addition of 1% chitosan concentration in the biscuit formulation containing red and purple roselle extract not only gave a significant contribution in improving the proximate composition of the protein and fat content of the biscuits but also more effectively increased the phenolic content and antioxidant activity. The addition of 1% chitosan to the biscuit formulation containing red (4% and 6%) and purple (4% and 6%) roselle extracts also had a good acceptance by the panelists of aroma, colour, texture, taste, and overall acceptance of the biscuits. Biscuit samples containing purple roselle extract also showed greater total phenolic and antioxidant activity than biscuits with ingredients containing red roselle. These biscuits can be consumed as alternative snacks that have high antioxidant value and nutritional content, hence they are useful for increasing body resistance.

Conflict of interest

The authors declare no conflict of interest.

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